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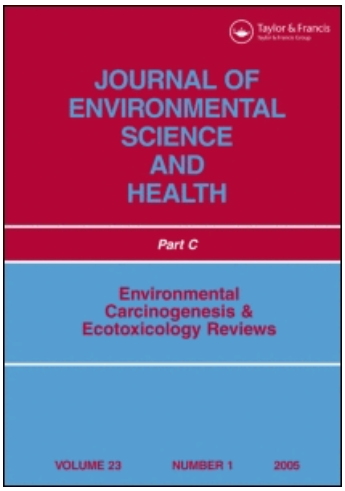

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8-hydroxy-2' -deoxyguanosine (8-OHdG): A Critical Biomarker of Oxidative Stress and Carcinogenesis

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8-hydroxy-2′-deoxyguanosine (8-OHdG): A Critical Biomarker of Oxidative Stress and Carcinogenesis

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There is extensive experimental evidence that oxidative damage permanently occurs to lipids of cellular membranes, proteins, and DNA. In nuclear and mitochondrial DNA, 8-hydroxy-2′-deoxyguanosine (8-OHdG) or 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) is one of the predominant forms of free radical-induced oxidative lesions, and has therefore been widely used as a biomarker for oxidative stress and carcinogenesis. Studies showed that urinary 8-OHdG is a good biomarker for risk assessment of various cancers and degenerative diseases. The most widely used method of quantitative analysis is high-performance liquid chromatography (HPLC) with electrochemical detection (EC), gas chromatography-mass spectrometry (GC-MS), and HPLC tandem mass spectrometry. In order to resolve the methodological problems encountered in measuring quantitatively 8-OHdG, the European Standards Committee for Oxidative DNA Damage was set up in 1997 to resolve the artifactual oxidation problems during the procedures of isolation and purification of oxidative DNA products. The biomarker 8-OHdG or 8-oxodG has been a pivotal marker for measuring the effect of endogenous oxidative damage to DNA and as a factor of initiation and promotion of carcinogenesis. The biomarker has been used to estimate the DNA damage in humans after exposure to cancer-causing agents, such as tobacco smoke, asbestos fibers, heavy metals, and polycyclic aromatic hydrocarbons. In recent years, 8-OHdG has been used widely in many studies not only as a biomarker for the measurement of endogenous oxidative DNA damage but also as a risk factor for many diseases including cancer.

Key Words: Reactive oxygen species; biomarker; oxidative damage; DNA; 8-OHdG; 8-oxodG; carcinogenic substances; carcinogenesis

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INTRODUCTION

Reactive oxygen species (ROS) are formed continuously in living cells of aerobic organisms as part of the physiological processes, metabolic, and other biochemical reactions. These endogenously produced ROS and oxygen-free radicals have important physiological functions, but because of their reactive nature can cause oxidative damage to lipids of cellular membranes, proteins, and DNA (1). Also, exogenous factors, such as UV radiation, tobacco smoke, asbestos, and carcinogenic substances, can produce ROS under various conditions (2)

Under normal physiological conditions in all aerobic organisms, there is a balance maintained between endogenous oxidants and numerous enzymatic and non-enzymatic antioxidant defenses (3,4). When an imbalance occurs, oxidants produce extensive oxidative damage to DNA, which, in turn, contributes to aging, malignant tumors, and other degenerative diseases (5–7).

In all living cells, damaged DNA is repaired enzymatically so that they regain their normal function, whereas misrepaired DNA can result in mutations (base substitution, deletions, and strand fragmentation) leading to carcinogenesis (8, 9). Although a broad range of DNA products are produced during oxidative damage to DNA (bases and sugar modifications, covalent crosslinks, single- and double-stranded breaks), most interest focused on nucleobase modifications and especially on the abundant lesion of 8-oxo-2′-deoxyguanosine because it is formed in vivo and can be measured quantitatively in cells following hydrolysis of the DNA to component bases (10).

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Reactive Oxygen Species and Oxidative DNA Damage

The most important oxygen-free radical causing damage to basic biomolecules (proteins, membrane lipids, and DNA) is the hydroxyl radical ($HO^{\bullet}$). The hydroxyl radical can be produced by various mechanisms, especially by the Fenton reaction of hydrogen peroxide (which diffuses into the nucleus) and metals and other endogenous and exogenous ROS. The $HO^{\bullet}$ attacks DNA strands when it is produced adjacent to cellular and mitochondrial DNA causing the addition of DNA bases new radicals, which lead to the generation of a variety of oxidation products (11).

The interaction of $HO^{\bullet}$ with the nucleobases of the DNA strand, such as guanine, leads to the formation of C8-hydroxyguanine (8-OHGua) or its nucleoside form deoxyguanosine (8-hydroxy-2′-deoxyguanosine). Initially, the reaction of the $HO^{\bullet}$ addition leads to the generation of radical adducts, then by one electron abstraction, the 8-hydroxy-2′-deoxyguanosine (8-OH-dG) is formed (Fig. 1). The 8-OHdG undergoes keto-enol tautomerism, which favors the oxidized product 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG). In the scientific literature 8-OHdG and 8-oxodG are used for the same compound (12).

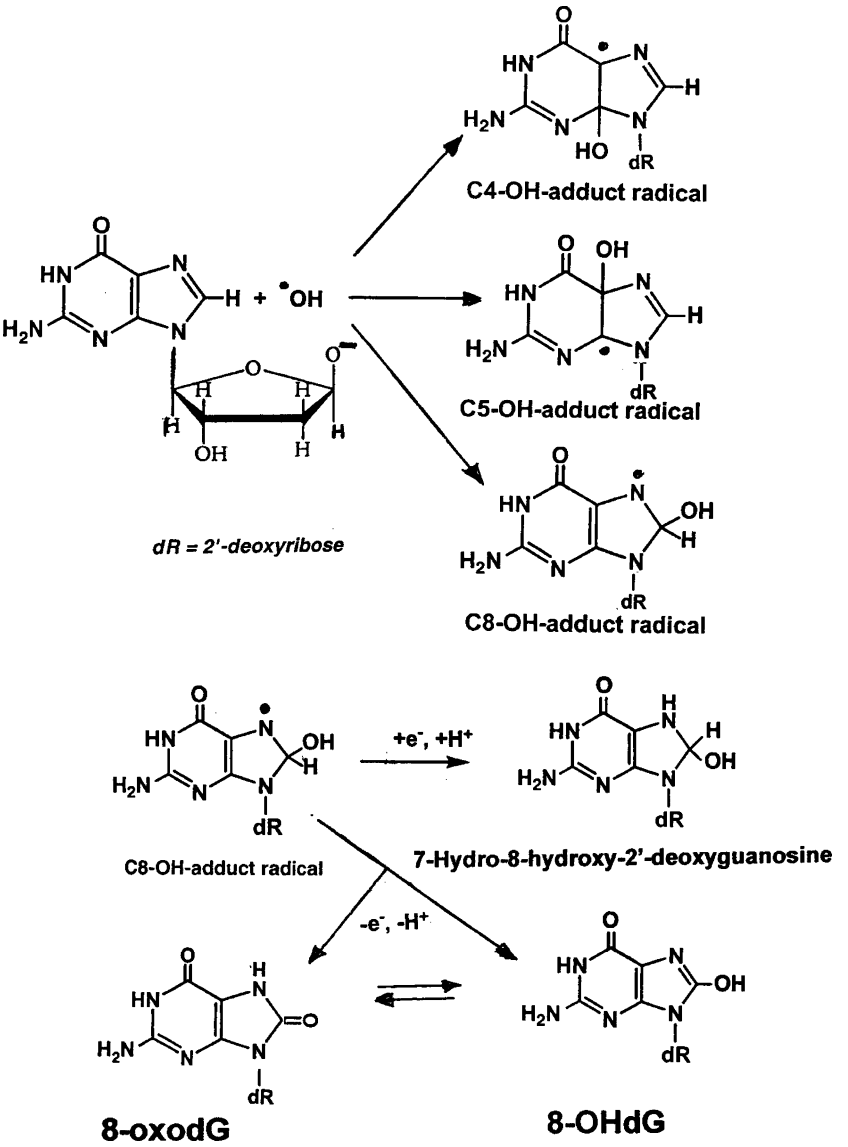

Figure 1: Reaction of 2′-deoxyguanosine with hydroxyl radicals, radical adducts followed by reduction to 7-hydro-8-hydroxy-2′-deoxyguanosine, and by oxidatioin to 8-hydroxy-2′-deoxyguanosine (8-OHdG) or its tautomer 8-oxo-7-hydro-2′-deoxyguanosine (8-oxodG).

Although the other nucleobases of DNA react with HO• in a similar manner, the 8-oxodG lesion is the most abundant DNA lesion because it is relatively easily formed and is promutagenic, and therefore a potential biomarker of carcinogenesis (12). Experiments showed that the mutagenic potential of 8-oxodG is supported by a loss of base-pairing specificity, misreading of adjacent

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pyrimidines, or insertion of adenine opposite the lesions (13). Mutations that may arise due to the formation of 8-oxodG involving GC → TA transversion mutations (10, 14).

In recent years, the 8-OHdG lesions can be detected and analyzed with high sensitivity by high-performance liquid chromatography (HPLC), gas-chromatography-mass spectrometry (GC-MS), and liquid chromatography-mass spectrometry-mass spectrometry (LC-MS-MS) and by immunohistochemical methods and single cell gel electrophoresis. Determination and analysis of 8-OHdG can be performed in animal organs and in human samples (urine, human organs, leukocyte DNA) as a biomarker of oxidative stress, aging, and carcinogesis (15).

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Key Stages of the 8-OHdG Discovery

The discovery of 8-OHdG was first reported in by Kasai and Nishimura in 1984 in their attempt to study and isolate mutagens in heated glucose (as a model of cooked food). Because of the difficulty to isolate mutagens, which are very unstable, they developed a method to trap reactive mutagens as guanine derivatives from the fact that carcinogens and mutagens react with nucleic acid bases, particularly guanine (16). The same authors found that oxygen-free radicals are involved in the C-8 oxidation reaction (17). In the following years, the formation of 8-OHdG was confirmed under exposures/reactions generating oxygen free radicals, such as asbestos fibers and H_2O_2. In the second case, H_2O_2 and ferrous ions used a reducing agent (Fenton reaction) (18, 19) as did the carcinogenic cigarette smoke and diesel exhaust particles (20, 21)

In 1986, Floyd and coworkers (22) reported an analytical method for sensitive detection for 8-OHdG in cellular DNA by HPLC with electrochemical detector (HPLC-EC). The method was used by various scientific groups for numerous analytical studies of DNA damage in animal organs after the administration of various carcinogenic chemical substances and tumor promoters (23–26).

Similarly, numerous papers in recent years have analyzed levels of 8-OHdG in human organs and leukocyte DNA and in urine in relation to oxidative stress, diet, cancer incidence, and aging. As a result of these studies 8-OHdG has been established as an important biomarker of oxidative stress (12, 27), of cancer risk to humans by mechanisms of oxygen-free radicals (28, 29), of aging processes including degenerative diseases (30, 31), and in general as a biological marker of lifestyle and the effect of diet (32, 33).

In the past 20 years, numerous papers appeared in the scientific literature on the subject of oxidative stress and oxidative DNA damage, the role of oxygen-free radicals and ROS, and 8-OHdG or 8-oxodG. A search of Scholar Google in the end of 2008 showed 56,300 papers on oxidative DNA damage, 6,180 for 8-OHdG, and 2,430 for 8-oxodG.

Methodology and Problems for the Analysis of 8-OHdG

The first reported analysis of 8-OHdG as a major and ubiquitous oxidation product of DNA in experimental animals in the urine and in humans was performed in 1989 (27). Since then several scientific groups have reported the 8-OHdG (or 8-oxodG) analysis by various methods.

The methodology of analysis followed two different strategies: (1) the direct approach designated to single out the DNA lesion by using physical and chemical methods and final DNA extraction and hydrolysis, and (2) the indirect approach, by preserving the whole DNA structure and the formation of the lesions are monitored in situ (34). In the first approach the quantitative analysis is performed by HPLC coupled with electrochemical detection (HPLC-EC), gas chromatography-mass spectrometry (GC-MS), and by HPLC tandem mass spectrometry (HPLC-MS/MS) (35–37). In the second approach the measurement is performed either by using antibodies that generally exhibit low specificity or through nicking activity of a specific DNA repair enzyme (38). Also, by using the enzyme formamidopyrimidine DNA N-glycosylase, the 8-oxodG is converted into a strand break and the quantitative measurement of the strand breaks is estimated by the comet assay, the alkaline elution technique, or the unwinding method (39–41).

Methodological differences among scientific laboratories directly analyzing the levels of 8-oxodG have resulted in a lack of consistency in the results, especially in the exaggeration of the background levels of 8-OHdG in human cellular DNA, sometimes by up to two or three orders of magnitude (42, 43). Although there are variations of levels between cell types and animal organs, such large differences were attributed to inaccurate experimental protocols and isolation methodologies that give rise to overestimations (probably by artefactual DNA oxidation during the procedure). As a general trend, levels of 8-oxodG obtained by the indirect approach (enzymatic assays) were found significantly lower than those of the direct methods of measurements (34).

The European Standards Committee on Oxidative Damage was set up in 1997 to resolve these issues (supported by the European Commission as a Concerted Action) with 27 analytical laboratories as members. The participating laboratories were provided with standard solutions of 8-oxoGua/8-oxodG in samples of calf thymus DNA, which required hydrolysis either to nucleosides using DNA-degrading enzymes (prior to analysis by HPLC) or to bases with formic acid (procedure normally used for GC-MS analysis) (44). Three samples were analyzed by all laboratories in triplicate by HPLC, GC-MS, and LC-MS-MS against their own standard. The results showed that the analysis of 8-oxodG in standard solutions was more accurate than previous analyses (15). The method LC-MS-MS was found to be demonstrably as reliable, sensitive, and precise as the best HPLC procedures and had the added advantage of giving unambiguous information on the identity of analytes. The HPLC

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with electrochemical detection (EC-coulometric version) is more sensitive than the EC-amperometric version. The Committee noticed some problems with the GC-MS method, which resulted in higher values of 8-oxoGua as a result of oxidation occurring during sample preparation.

The direct approach of measurements, which requires isolation from cells of tissues, followed by hydrolysis and the release of the DNA lesions, has to be quantified by very sensitive detection techniques at the output of a chromatographic column. Thus, in each of the individual steps, artifactual DNA oxidation may occur, leading to overestimated levels (45, 46).

Cadet and colleagues (34) used a series of cell treatments methods, various digestion protocols, and analytical techniques to establish the cellular background level of 8-oxodG in cells. Their results showed that the steady-state cellular background level of 8-oxodG, in a lymphocyte cell line, was ~0.5 lesions/10^6 DNA nucleosides.

The levels of 8-oxodG were measured in DNA and compared under different methods, using HPLC-EC, EPLC-MS/MS, and HPLC-GC-MS analytical techniques (47–49). In recent years more sensitive analytical techniques used for the measurements and background levels of 8-oxodG in human urine were found at around 1 lesion/10^6 DNA nucleosides (50–51)

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ENDOGENOUS OXIDATIVE DNA DAMAGE

The Levels of 8-OHdG and Endogenous Oxidative DNA Damage

It was known that cellular (cDNA) and mitochondrial DNA (mtDNA) in aerobic organisms are constantly damaged even in the absence of any exposure to genotoxic carcinogens. Damaging processes include endogenous sources, such as DNA instability (e.g., depurination), spontaneous errors during DNA replication and repair, numerous physiological ROS as a result of oxygen metabolism, and products of lipid peroxidation. Exogenous sources also contribute to DNA damage, such as ionizing and UV radiation, naturally occurring radioisotopes, numerous genotoxic phytochemicals, and food pyrolysis products in diet and contaminants of air and water. Although aerobic biological systems are protected by antioxidant enzymatic and nonenzymatic antioxidants and by special DNA repair mechanisms that reduce substantially the levels of damage, a certain steady-state level is measurable as a background at all times (53).

Attempts to measure and quantify levels of background DNA damage were made in the 1980s (54, 55). The remarkable progress made in the next 10 years with respect to analytical sensitivity, structural characterization, and quantification of DNA lesions resolved many aspects of the problem. Oxidative stress, oxygen-free radicals, and lipid peroxidation products as byproducts of aerobic

metabolism were studied as genotoxic agents of endogenous DNA damage and were presented in a special issue of *Mutation Research* (56–59).

The etiology of various types of cancer is the result of DNA modifications and replication errors in somatic cells that give rise to mutations and subsequently to promotion and development to the malignant tumor. The basal steady-state levels of oxidative DNA base modifications (such as 8-oxodG) can be found in all types of cells, mostly from the continuous generation of ROS in the cellular oxygen metabolism (60). Scientists have long suspected that this endogenous basal DNA damage might play an important role in the initiation of carcinogenesis, whereas the environmental-exogenous effects are thought to mostly influence the stage of tumor promotion. The high incidence of sporadic cancer that is actually seen in our population can hardly be explained by the level of exposure to exogenous carcinogens of environmental pollution (61, 62). The hypothesis of the role of endogenous DNA damage by ROS attracted lots of attention by other scientists (8, 63, 64).

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The correlation of the steady-state levels of oxidative DNA damage (ratio of the endogenous generation rate of DNA damage and the rate of the repair of these modifications, taking into account the cellular antioxidants and the efficiency of the repair system) with the mutation frequencies and the cancer incidence depended on the availability of sensitive methods of analysis of low levels of DNA damage. Since the 8-oxodG was the most frequently determined biomarker for the oxidative DNA damage its quantification at low levels was very important to test the model for the correlation (65).

The magnitude of endogenous DNA damage by ROS may be enormous. Each human cell metabolizes approximately 10^{12} molecules of oxygen per day with 1% of oxygen metabolism, resulting in the generation of ROS (66). Based on the urinary excretion of characteristic modified nucleosides produced by ROS damage to DNA (8-oxodG and thymine glycol), it has been estimated that approximately 20,000 nucleobases in DNA are damaged in each human cell per day (67). The high level of oxidative damage by endogenous ROS was supported by Ames and colleagues, especially in the case of micronutrient deficiencies in humans (68, 69).

Although some scientists were doubtful as to the high levels of basal oxidative DNA damage, the quantitative estimates of steady-state levels of 8-oxodG, determined by various techniques in mammalian cells, ranged over several levels of magnitude. We now know that this was due to discrepancies in experimental procedures. The most recent reported estimates of steady-state levels of 8-oxodG in the DNA of human lymphocytes by various techniques were in the range of 0.2–2.0 lesions detected per 10^6 nucleobases (47).

Relevance of Endogenous DNA Damage in Carcinogenesis

The relevance of endogenous DNA damage by ROS in carcinogenesis is a very difficult problem, and scientists tried to devise methods to estimate its

contribution. The in vivo studies have to overcome the additional influence of inflammation, which increases the level of oxidative damage and contributes to the increase of progression to cancer (64, 70). Also, epigenetic effects by ROS and oxidative stress, such as various types of signal transduction mechanisms, lead to apoptosis, modulate cell proliferation, and immune response, thus complicating the estimation of risk (71–73). Studies with antioxidants, such as vitamin C, β-carotene, and α-tocopherol, for in vivo experiments showed inconsistent results. Dietary supplementation of the various antioxidants does not necessarily improve the antioxidant capacity of the cells and effect the steady-state levels of 8-oxoG or 8-oxodG in human lymphocytes in a human volunteer study (74) or in the liver of guinea-pigs (75).

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Another approach of scientific research to solve the problem was to study the influence of DNA repair mechanisms on modulation of oxidative DNA damage. It is known that DNA base modifications are recognized and removed in eukaryotic cells by specific glycosylases that initiate the base excision repair pathway. For the lesion 8-oxoG, the most important and relevant enzyme in mammalian cells is the Ogg1 protein, in which the gene was cloned (76, 77). Overexpression of Ogg1 in cultured cells increased the repair rate by several fold but did not reduce the steady-state level or the spontaneous mutation rate significantly (78). Various scientific groups used the $ogg1^{-/-}$ knockout mice as a tool to analyze the effect of decreased DNA repair rates in vivo. Studies showed that there is an increase of the steady-state levels of 8-oxoG with the resulting increases of the spontaneous mutation frequencies observed in the liver of $ogg1^{-/-}$ mice (79–81). Results showed an increase of less than five additional 8-oxoG residues per 10^6 bp (double the spontaneous mutation rate). Therefore, 8-oxoG seems to play a major role for spontaneous mutagenesis (60).

The contribution of mitochondria to endogenous DNA damage of the nucleus, where the electron respiratory transport chain is the most important source of ROS in mammalian cells, was investigated by Epe and colleagues. The results showed that the contribution of mitochondria to the endogenously generated background levels of oxidative damage in the nuclear DNA is negligible (82).

PRACTICAL APPLICATIONS OF THE 8-OHdG BIOMARKER

Measurements of the 8-OHdG as a Biomarker in Relation to Exposures to Carcinogenic Substances

Analysis of urinary 8-OHdG has been established as an important biomarker to evaluate oxidative stress and to assess risk to cancer after exposure to various carcinogenic substances, environmental pollutants, and lifestyle factors (32, 83).

Tobacco smoking is well known for its oxidative properties and its carcinogenic potential (84). Elevated levels of 8-oxodG have been found in human tissues, including lungs and peripheral leukocytes of smokers (85, 86). Also, increased levels of 8-OHdG have been found in passive smokers in the workplace exposed to environmental tobacco smoke (87). Environmental tobacco smoke (ETS) can be a possible cancer risk factor in offspring of experimental animals. Exposure of pregnant rats to a relevant dose of 1 mg/m^{3}of ETS during gestation resulted in a significant increase in 8-oxodG in maternal liver and in fetal liver and brain (88). The urinary excretion of products of damaged nucleotides in cellular pools or in DNA has been proved to be an important biomarker of risk for lung cancer. A cohort of 53,689 (men and women) aged 50–64 years, with 3–7 years follow-up, including 260 cases with lung cancer, were studied. Urinary excretion of 8-oxodG was higher in current smokers. Overall the incidence rate ratio (95% confidence interval) of lung cancer was 0.99 (0.80–1.22) per doubling of 8-oxodG excretion and there was no interaction with Ogg1 genotype (89).

Asbestos fibers have been proved to be genotoxic in the lungs and are a potential carcinogenic hazard to occupationally exposed workers. Preliminary studies in cell free systems and in cultured cells showed that asbestos fibers produce hydroxyl radicals and cause oxidative DNA damage and act synergistically with cigarette smoke (90, 91). Other studies showed that inhaled asbestos fibers induce the formation of 8-OHdG in the DNA of white blood cells of workers highly exposed at the workplace (92, 93); high levels were found in the urine of asbestos-exposed workers, which correlated positively with duration of exposure (94).

Diesel exhaust particles and fine particulates, a mixture of organic chemicals, heavy metals, and carbon particles with persistent free radicals, are known by various studies to have the ability to cause oxidative injury and are potentially carcinogenic. Studies in mice showed a positive correlation of levels of 8-OHdG and lung carcinogenesis (95). In recent years many studies were devoted to studies of the importance of levels of urinary 8-OHdG in human lung tissues of workers exposed to diesel fumes or oil fly ash (96–98).

Ambient particulate air pollution in urban environments has been associated with an increase in lung cancer and cardiopulmonary mortality (99). The role of free radicals and other ROS formed by particulate matter (PM) has been linked to their increased toxicity in lung tissues (100). The biomarker 8-OHdG was used to study the effects of hydroxyl radical (HO$^\bullet$) generated both by coarse and fine PM (especially PM with aerodynamic diameter 10–2.5 μm, PM_{10} and $PM_{2.5}$) due to the heavy metals adsorbed in the pores and surfaces of the particles (101). Heavy metal content (Cu, Fe, V, etc.) of particulate matter and their bioreactivity was established as an important contributory factor to the enhancement of oxidative DNA damage (102, 103).

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There are limited studies on the enhancement of urinary excretion of 8-oxodG or in the nasal respiratory epithelium in human subjects exposed to increased urban particulate air pollution, such as bus drivers and students working and living in central Copenhagen, children in Mexico City, and female toll station workers in Taipei (Taiwan) (104–107).

The correlation of inhaled particulate air pollution and oxidative DNA damage mechanisms by ROS (measured as 8-oxodG) or by stimulating cellular oxidant generation, has been recently reviewed (108–110).

Heavy metals

Heavy metals and some metalloids are known to be carcinogenic to humans (111). The oxidative concept in metal carcinogenesis proposes the involvement of redox mechanisms, the production of hydroxyl radicals, which can attack DNA, causing hydroxylation and oxidative DNA damage. In turn, some of these products of DNA damage (including 8-oxodG) induce mutations leading to neoplastic transformations (112). Many studies investigated the carcinogenic potential of various metal ions via ROS generation (113, 114) and the induction of 8-OHdG lesion (115–117).

Polycyclic aromatic hydrocarbons (PAHs) have been known from epidemiological studies to be carcinogenic to humans. Elevated levels of 8-OHdG in urine or white blood cells of workers exposed to PAHs were determined for different working environments (e.g., coke-oven, roofers exposed to coal-tar pitch dust, etc) (118–121).

Various studies have investigated the effects of known carcinogens, such as benzene, styrene, and inorganic arsenic, using the urinary biomarker of 8-oxodG in workers exposed separately in different types working environments. The results of these studies showed a dose-response effect (122–124). A comprehensive review of the use of 8-OHdG as a marker of oxidative DNA damage in occupational and environmental exposures to carcinogenic substances was recently published (125).

Epidemiological Evidence for Carcinogenic Risk and 8-OHdG

In recent years, the application of the biomarker 8-OHdG or 8-oxodG in molecular epidemiology studies as a risk factor for many diseases (especially cancer) associated with oxidative stress mechanisms is increasing (126). In the following section we present selected results only for recent years.

Elevated levels of urinary levels of 8-OHdG has been detected in patients with various malignancies. Chinese scientists measured urinary 8-OHdG in children with acute leukemia; their results suggested that elevated levels were associated with oxidative DNA damage. Furthermore, it was found that the risk was correlated with high levels of Cr exposure (127). Serum 8-OHdG was significantly increased in patients with colorectal cancer compared with

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the control group. Also, the authors found that the activities of antioxidant enzymes in the study group were also significantly reduced (128). A recent study from Japan investigated the increased production of ROS and oxidative DNA damage in relation to hepatocarcinogenesis. Multivariate analysis found that levels of 8-OHdG and fibrosis were significant risk factor for hepatocellular carcinoma, especially in patients with hepatitis C virus infection (129).

Salivary analysis of 8-OHdG biomarker in patients with oral squamous cell carcinoma showed an increase of 65% compared with controls (130). Weight loss has been shown to increase oxidative DNA damage, thus increasing risk of some cancer forms, including lung cancer. An epidemiological study with 174 healthy employees showed that one unit decrease of Body Mass Index was associated with a 2.7% increase in 8-OHdG levels (131). Urinary 8-OHdG levels were also significantly higher among breast cancer patients than among control subjects, after making adjustments for confounders such as coffee consumption and use of oral contraceptives (132). Oxidative DNA damage, in the form of 8-OHdG levels, was found elevated for patients with Barett's oesophagus and adenocarcinoma of oesophagus (133)

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CONCLUSIONS

Reactive oxygen species, including oxygen and nitrogen-free radicals, can cause specific oxidative DNA damage and play a leading role in initiation and promotion of carcinogenesis. The biomarker 8-OHdG or 8-oxodG, which was first reported by Kasai and Nishimura in 1984, has been established as a commonly measured and sensitive marker of DNA damage due to the hydroxyl radical attack at the C8 position of the nucleobase guanine or its nucleoside guanosine. Results showed that this damage, if left unrepaired, may contribute to mutageninicity and cancer promotion. Many studies in the past 20 years and improvements in the quantitative estimation of 8-OHdG by various analytical techniques in blood cells or in urine have established it as a very important biomarker not only for carcinogenesis but also for aging and degenerative diseases.

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